

## AMENDMENTS/CURRENT LISTING OF CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

### Listing of Claims:

Claim 1 (currently amended): A method for determining candidates for gene deletions and additions using a model of a metabolic network associated with an organism, the method comprising:

providing a model in a computer comprising an electronic representation of a plurality of metabolic reactions defining metabolite relationships;

selecting at least one bioengineering objective function for the organism;

selecting at least one cellular objective function for the organism;

forming a linear optimization problem that couples the at least one cellular objective function with the at least one bioengineering objective function;

executing commands in a suitably programmed computer for ~~simultaneously~~ solving the linear optimization problem to provide an optimal solution for the bioengineering objective function that simultaneously requires optimality of the cellular objective function and that yields at least one candidate gene deletion; and

providing a visual output of the at least one candidate gene deletion to a user.

Claim 2 (previously presented):      The method of claim 1 further comprising using the at least one candidate to genetically modify the organism.

Claim 3 (previously presented):      The method of claim 1 wherein the bioengineering objective function is overproduction of a chemical.

Claim 4 (previously presented):      The method of claim 1 wherein the bioengineering objective function is underproduction of a chemical.

Claim 5 (previously presented):      The method of claim 1 wherein the cellular objective function is growth.

Claim 6 (withdrawn): The method of claim 1 wherein the cellular objective is minimization of metabolic adjustment.

Claim 7 (original): The method of claim 1 wherein the candidate is a candidate for gene deletion, and the optimization problem includes a binary value for specifying if reaction flux is active or inactive.

Claim 8 (original): The method of claim 1 wherein the optimization problem is a bilevel optimization problem.

Claim 9 (withdrawn): The method of claim 1 wherein the optimization problem is a mixed-integer optimization problem.

Claim 10 (original): The method of claim 1 wherein the optimization problem includes at least one stoichiometric constraint.

Claim 11 (original): The method of claim 1 wherein the optimization problem includes at least one chemical uptake constraint.

Claim 12 (previously presented): The method of claim 1 wherein the step of forming an optimization problem includes quantifying the cellular objective function as an aggregate reaction flux.

Claim 13 (previously presented): The method of claim 1 further comprising evaluating performance limits of the metabolic network with the at least one candidate based on ability of the network to meet the at least one cellular objective function.

Claim 14 (previously presented): The method of claim 1 wherein the cellular objective function is selected from the group consisting of: maximizing a growth rate, maximizing ATP production, minimizing metabolic adjustment, minimizing nutrient uptake, minimizing redox production, minimizing a Euclidean norm, and combinations thereof.

Claim 15 (withdrawn): The method of claim 1 wherein the bioengineering objective is overproduction of glycerol and at least one candidate is for gene deletion and comprising genes coding for the enzymes fructose- 1,6-bisphosphatase, fructose- 1,6-bisphosphatase aldolase, phosphoglycerate kinase, glyceraldehydes-3-phosphate dehydrogenase, phosphoenolpyruvate synthase, NADH dehydrogenase I, phosphogluconate dehydratase, 2-keto-3-deoxy-6-phosphofluconate aldolase, triosphosphate isomerase, glucose 6-phosphate-1 -dehydrogenase, 6-phosphogluconolactonase- , deoxyribose-phosphate aldolase, aldehyde dehydrogenase, or combinations thereof.

Claim 16 (withdrawn): The method of claim 1 wherein the bioengineering objective is overproduction of 1,3-propanediol and at least one candidate is for gene deletion and comprising genes coding for the enzymes fructose- 1,6-bisphosphatase, fructose- 1,6-bisphosphatase aldolase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosphosphate isomerase, glucose 6-phosphate-1 -dehydrogenase, 6-phosphogluconolactonase, deoxyribose-phosphate aldolase, aldehyde dehydrogenase, or combinations thereof.

Claim 17 (withdrawn): The method of claim 1 wherein the bioengineering objective is overproduction of succinate and at least one candidate is for gene deletion and comprising genes coding for the enzymes pyruvate formate lyase, acetaldehyde dehydrogenase, pyruvate kinase, FOF1-ATPase, NADH dehydrogenase I, fumarase, D-Lactate dehydrogenase, pyridine nucleotide transhydrogenase, phosphotransacetylase, acetate kinase, phosphotransferase, or combinations thereof.

Claim 18 (previously presented): The method of claim 1 wherein the bioengineering objective function is overproduction of lactate and at least one candidate is for gene deletion and comprising genes coding for the enzymes phosphotransacetylase, acetate kinase, phosphofructokinase, fructose-4 1,6-bisphosphatase aldolase, triosphosphate isomerase, acetaldehyde dehydrogenase, glucokinase, or combinations thereof.

Claim 19 (currently amended): A computer-based method for determining candidates for gene deletions using a model of a metabolic network associated with an organism, the method comprising:

providing a model in a computer comprising an electronic representation of a plurality of metabolic reactions defining metabolite relationships;

inputting at least one bioengineering objective function;

receiving as input as least one cellular objective function;

forming a linear optimization problem that quantifies the at least one cellular objective function as an aggregate reaction flux and couples the at least one cellular objective function with the at least one bioengineering objective function;

executing commands in a suitably programmed computer for ~~simultaneously~~ solving the linear optimization problem to provide an optimal solution for the bioengineering objective function that simultaneously requires optimality of the cellular objective function and that yields at least one candidate gene deletion; and

visually outputting the at least one candidate gene deletion to a user.

Claim 20 (previously presented): The method of claim 1 wherein the cellular objective function comprises maximizing a growth rate.

Claim 21 (previously presented): The method of claim 1, further comprising solving the linear optimization problem to yield at least one candidate gene addition.

Claim 22 (previously presented): The computer-based method of claim 19, further comprising solving the linear optimization problem to yield at least one candidate gene addition.